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16. ABSTRACT

This book chapter considers the nature and properties of microcosms, the variability of the subsurface environment as it affects investigations with microcosms, microcosms as physical models, and the rational design of microcosms. It also considers the properties and appropriate use of several microcosms, including (1) two microcosms to study survival of microorganisms in ground water, (2) a microcosm to study biotransformations of concentrated industrial wastes after deep-well disposal, (3) a microcosm for the study of biotransformation of pollutants which uses internal recirculation of water to allow repeated sampling, (4) a microcosm in column configuration to study biotransformations of pollutants which are highly volatile or sorb strongly to rubber or plastic, and (5) a simple batch microcosm to study biotransformations of pollutants that are volatile.

17. KEY WORDS AND DOCUMENT ANALYSIS

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CHAPTER 7 - MICROBIAL ACTIVITY IN MODEL AQUIFER SYSTEMS

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7.1 Nature and Properties of Microcosms

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7.1 Nature and Properties of Microcosms.

The word microcosm comes from the Greek for "little world." For purposes of this chapter, we shall use the term very broadly to refer to any experimental system in which various components of the subsurface environment are isolated and then studied as the parameters of interest change with time. The approach contrasts with repeated sampling of the original environment to monitor its behavior.

Ideally, a natural system should be studied directly, and in its entirety, so that the components, processes, and interactions that comprise its structure and function can be described in a holistic and integrated manner. Unfortunately, constraints on physical and human resources make such an approach infeasible in many natural systems. This is particularly true for the terrestrial subsurface. The cost of drilling sampling-wells or bore holes can be inordinately expensive. Fortunately, the use of microcosms allows detailed study of the processes occurring in the subsurface environment without the need for a large number of wells or bore holes. A second advantage of microcosms is their flexibility. The components of a microcosm can be tailored to focus on particular processes or interactions. As a result, the design of a microcosm is usually keyed to the purpose of a particular study.

Microcosms fall into two broad classes. Those in the first class are designed to reveal the effects of an environmental perturbation on the composition and functioning of the biological community. The focus is on the biota. Little work has been done that is relevant to regions deeper than the root zones; however, the subsurface microflora may affect the quality of ground water in ways that are not appreciated at present, and their importance in the overall functioning of the biosphere should be investigated.

Microcosms in the second class are used to define the processes that affect the transport and fate of specific environmental pollutants, i.e. synthetic organic chemicals, heavy metals, pathogens, radionuclides, salts, and heat.

The focus is on the pollutant.

There are two approaches to the second class of microcosms. The first approach is empirical. The behavior of the microcosm is considered a simulation of the larger system. Ideally, the microcosm contains all the components of the larger system and exhibits processes and interactions that parallel the larger system. This approach is commonly used when the goal is to define the processes that occur in the subsurface environment, but not necessarily to describe them precisely.

The second approach uses the microcosm (here more properly called a physical model) to obtain values required to solve mathematical equations which describe the microcosm and are used to simulate the larger natural system. If the equations are considered hypotheses that are tested by their ability to predict the behavior of other microcosms operated under new conditions, or more preferably the larger natural system, the approach has the structure of science. If, however, the equations are tested by their ability to explain the behavior of the same physical models that were used to supply the necessary values, the process reduces to curve fitting and the validity of the assumptions in the equation has not been subjected to a proper scientific test.

The first approach is the quickest and cheapest way to get started on a problem. However, the results depend on the faithfulness of the simulation and have limited generality. The second approach requires more time and is more expensive, particularly if the mathematical models must be validated against the larger system, but the predictions are much more general.

7.2 Variability in the Subsurface Environment as It Affects Investigations Using Microcosms.

There are several kinds of variation within the subsurface environment that can complicate the extrapolation of data gained in microcosms to larger natural systems. This variation can be either temporal or spatial. Temporal

variation may reflect recent precipitation, seasonal changes, or shifts in climate, depending upon the rate of recharge. Spatial variation reflects both the distribution of point sources of pollution about the landscape, as well as variations in the geological composition of subsurface materials. In comparison to natural systems on the surface, the subsurface is strongly three-dimensional. Processes in the subsurface may vary profoundly with depth.

If the information needed is very site-specific and the site is relatively uniform, samples can be taken from the whole site, mixed and used to fill replicate microcosms. In this way variability between microcosms will be reduced and, if enough subsamples are taken initially to represent the whole site, the data will be representative of the area. If on the other hand, the structural integrity of the sample must be maintained or if minimum contamination of the sample by foreign microorganisms is desired, undisturbed cores may need to be taken from the smallest possible area to minimize variability.

Organisms sorbed onto solid material will be subjected to a changing environment as ground water moves through the matrix. A recent study has been chosen as an example of the changes in conditions experienced in a small area throughout a period of one year. Spalding and Exner (1) monitored a network of 53 wells ten times within one year. The principal aquifer consisted of Pleistocene sand and gravel. The ground water was unconfined, was recharged by river water, and moved at approximately 0.35 m/day. A secondary aquifer was confined by a clay deposit and consisted of lime-cemented silty sandstone of Tertiary age. Most of the study area was used for the production of irrigated corn, so that pollutants were probably moving down from the surface. Coefficients of variation from pooled variances for two parameters were calculated from their data for each set of wells. Results are shown in Table 7.1. There was

appreciable variation between wells in a relatively homogeneous area, and within the same wells at different seasons of the year. Shallow ground water showed higher variability of temperature since it is the immediate sink for vertically transported water originating in the vadose zone. Deep wells in the principal aquifer some distance away from the river showed less variation in chloride content than water from the secondary aquifer. Water temperature in both aquifers was considerably less variable than that taken from the river, although chloride levels were at least as variable.

In contrast to this natural variability most ground water microcosms kept in the laboratory to date have been maintained under conditions which were as constant as possible. (11, 14, 17, 18)

If a microcosm is used as a physical model, and a wide range of environmental conditions studied, extrapolation can be carried out over a large area. If, for example, it is hoped to apply the results to one rock type throughout the world, then the data given by Freeze and Cherry (2) for some parameters gives some indication of the range of conditions which would need to be tested to make the results generally applicable to one rock type. (Table 7.2)

As with most environments in the world, conditions in ground water range from those which permit growth of microorganisms to those which don't. For example, Dunlap and McNabb (3) have discussed the changes of temperature with depth. This ranges from a value which roughly corresponds to the mean annual air temperature of the given region at the water table to temperatures which would preclude microorganisms at a depth below about 2,500 m in areas of low geothermal activity.

7.3 Microcosms to Study Effects on the Biota.

Several simple microcosms have been constructed to study the rate of decline of populations of microcosms that have been introduced into aquifers as a result of waste disposal practices. Sinton (4) used a very simple microcosm

to identify microorganisms that could survive in an aquifer long enough to be useful as a tracer. These organisms could then be used to determine the probable movement of bacteria of sanitary significance.

Water was taken from wells and stored in the dark at 12.5°C in 5 l. flasks. Cultures of three strains of Escherichia coli were centrifuged and resuspended, then added to well water at densities between 10^8 and 10^9 cells/100 ml. The flasks were sampled 11 times over a period of 22 days. This flask microcosm has several deficiencies. The incubation temperature was the mean annual ground-water temperature of the area and did not duplicate fluctuations present in the natural ground water. Additionally, washing the cultures may not have removed all the nutrients in the culture medium, and autolysis of dead cells at the original high densities may have provided nutrients to the survivors, which would have increased their longevity relative to the natural aquifer. Finally, solid surfaces may enhance survival in the natural system, and the only surface available in this microcosm was the walls of the container. On the positive side, a flask microcosm is the ultimate in simplicity. It is easy to construct, easy to replicate, and easy to sample.

Martin and Noonan (5) designed a more complex microcosm to study the survival of natural populations of coliform bacteria in well water. Dialysis tubing was filled with ground water that had been contaminated by percolate from a municipal-waste disposal site. The tubing was hung inside a slotted PVC frame, and the assembly was lowered into the same well that provided the contaminated water. The assembly was raised periodically and sampled for faecal coliform bacteria.

A possible drawback of this microcosm could be a tendency for microorganisms to sorb to the walls of the tubing. However, the survival of the microbes is studied under more natural conditions. Using this microcosm, Martin and Noonan (5) determined that cell death and dilution, rather than sorption, were

primarily responsible for the reduction in numbers of faecal coliforms in ground water down-gradient from the disposal site.

McFeters et al. (6) used a more elaborate microcosm to compare survival of strains of pathogenic bacteria or the other faecal bacteria used as indicators of the pathogenic forms. Suspensions of bacteria were placed in chambers sealed with membrane filters (Fig. 7.1). The chambers were placed in a 40 l. tank that was replenished with well water at the rate of 5 l./min. The contents of the tank were mixed to ensure homogeneous conditions. The membrane filters probably allowed more rapid exchange between the microcosm and its surroundings than dialysis tubing. Additionally, the membrane would not exclude large organic polymers which might serve as food for the microorganisms.

McFeters et al. (6) showed that survival of pathogens and their indicator organisms in ground water at their study site were similar. Further, a comparison of survival times determined in the three microcosms discussed above reveals a good deal of consistancy between microcosms. (Table 7.3).

7.4 Microcosms that Reveal the Fate of Pollutants.

The impetus for the development of these microcosms was the need for information about specific pathways whereby wastes enter ground water, in order to facilitate intelligent environmental management and regulation. Attention has been directed toward deep injection wells for industrial wastes, toward materials leaching from landfills, and toward contaminant plumes from spills or other accidential releases of snythetic organic chemicals to the surface of the land.

Horvath and Elkan (7) designed a simple microcosm to study biological activity in a particular industrial waste under conditions pertaining to a deep saline aquifer. The microcosm was a glass column 8.5 cm I.D x 95 cm long, filled with sand and closed with a black rubber stopper (Fig. 7.2). The microcosm was autoclaved, then inoculated by pumping water from an uncontaminated well in the aquifer being simulated. The inoculum was protected from atmospheric oxygen, and drip tubes were used to prevent contamination of the microcosm by

laboratory microorganisms. The feed solution was a synthetic chloride-brine dosed with acetic, formic, para-toluic, terephthalic, phthalic and benzoic acids, methanol, and formaldehyde to simulate a particular industrial waste. The concentration of individual solutes varied from 52 to 9,359 mg/l. Feed solution under gravity flow was introduced into the bottom of the column. Any gas produced within the column was separated from the column effluent at the top of the column. Flow was controlled by metering the column effluent through a pump. Only formic acid was degraded in the microcosm. This result is in good agreement with the behavior of the genuine industrial waste after injection into a deep saline aquifer. Leenheer et al. (8) found that formate was completely degraded in the aquifer, acetate was degraded only slightly, and the other organic constituents were not degraded (methanol was not part of the injected waste).

This microcosm is well suited for study of anaerobic processes. Its components are cheap and easily obtainable. Because it does not require actual core material from the aquifer, the microcosm is inexpensive to set up and easily standardized. However, the microcosm has two limitations. The microflora within a well may be very different from the microflora of the aquifer _per se_ (See Chapter 12). Additionally, aquifer solids are rarely entirely sand. Low concentrations of natural organic matter present in almost all aquifers may be necessary for maintenance of the indigenous microflora. Organic matter and clays in the aquifer matrix may also sorb waste components and retard their movement.

This microcosm is well suited to define the biological fate of organic wastes present in concentrations great enough to enrich an active population (>10 mg/l, aerobically; >500 mg/l, anaerobically). Under these conditions, constraints on biodegradation imposed by a very low density or activity of the native microflora are not important. Use of rubber stoppers may present problems with certain reactive or very hydrophobic organic compounds, but in such a case, a Teflon closure can be substituted.

Horvath and Elkan (9) also described a simple microcosm that can be used to determine the effects of pressure on biodegradation. A screw-cap test tube is filled with liquid medium inoculated with the test organism. The tube is closed with a flexible diaphragm held in place by a perforated screw cap. The tube is inserted into a steel pressure chamber, and the remaining space is filled with water. Then a piston fitted to a screw is bolted to the chamber and the screw turned until the required pressure is reached. When a flora from a deep well (pressure at the well screen 32 atm) was examined in this microcosm for the ability to reduce nitrate and degrade formate, attack on these substrates was greater in the range of 34 to 100 atm. Because 100 atm corresponds to depth near 1,000 m, and few disposal wells exceed this depth, it is unlikely that pressure will limit biodegradation in most aquifers.

The device of Horvath and Elkan (9) can be adapted to any liquid or slurry that can be forced into a test tube. It would be particularly useful to test the barotolerance of laboratory cultures that are selected or genetically engineered for the degradation of waste in disposal wells or the biologically enhanced recovery of oil from oil fields.

Grula and Grula (10) constructed a similar microcosm that was pressured with compressed gas. When helium was used, their results were similar to those of Horvath and Elkan (9). When air was used, oxygen toxicity destroyed the microorganisms at relatively low pressures (16 atm).

Biodegradation in the subsurface may be limited by the concentration of electron acceptors (O_2, NO_3^{-1}, SO_4^{-2}). One remedy is to pump air into the aquifer. The device of Grula and Grula (10) could be used to determine the maximum depth at which such a remedy could work, or to select tolerant strains that could be used at greater depth.

Dunlap et al. (11) designed a unique microcosm to determine the fate of nitrilotriacetic acid (NTA) at environmentally relevant concentrations under conditions simulating as nearly as possible those prevailing in a natural aquifer (Fig. 7.3). Authentic aquifer material was collected from a shallow sandy aquifer in the flood plain of a river. Then 3.0 l. of aquifer matrix material was added to a 4.0 l. aspirator jar. A glass tube penetrated the mass of sand. Connected to the tube was a length of Tygon tubing that passed through a peristaltic pump and returned to the microcosm. The apparatus allowed internal circulation of water to facilitate sampling and to simulate ground-water flow. As far as possible, aseptic technique was employed in collection of the aquifer matrix material and in construction of the microcosm. Biodegradation was probably restricted to organisms originally present in the aquifer. Most of the native pore water was replaced with sterile solutions of Na_3NTA at an initial concentration of 30-55 mg/l. The headspace of the microcosm was filled with N_2; any gases evolved within the microcosm were sampled from the headspace. Microcosms were prepared in duplicate; there was also an autoclaved control.

Degradation of NTA was slow, 0.5-1.0 mg $l.^{-1}$ day^{-1}; there was no degradation in the autoclaved microcosm. Degradation was roughly zero order with concentration over the range of 50 to 5 mg/l; although there were some shifts in the rate of degradation that were probably due to changes in the density of active organisms. There was good agreement between the duplicate microcosms, even after 260 days of incubation and three additions of NTA.

This microcosm configuration has a number of advantages. It can be sampled repeatedly (within reason), because sampling is not destructive. As a result, the microcosm can be sampled intensively at the beginning of an assay, and then preliminary results used to schedule the remaining samples. This way a wide range of biodegradation rates can be adequately sampled. A second advantage is that the

entire sample of aquifer material has the same history and is subjected to the same concentration of test substrate. As a result, plots of concentration against time can be used to infer the rate law for degradation. This is often not the case with a column configuration.

The recirculating loop in the microcosm of Dunlap et al. (11) was made of Tygon, which might present problems of sorption of various organic compounds. However, Teflon-lined peristaltic tubing is now available and this microcosm could be constructed entirely of non-reactive materials. The large headspace in the microcosm would present formidable difficulties in the study of volatile organic compounds, and would also allow escape of carbon dioxide and methane produced by the microbes. Escape of carbon dioxide would tend to buffer the water in the microcosm from changes in pH that would occur in an aquifer. Because this microcosm is constructed with authentic aquifer matrix material, it is useful at lower concentrations of test compound that, although environmentally relevant, are too low to enrich an active population of degraders. It would be particularly useful in cases where the rate law and values for the rate constants for degradation are needed for mathematical modelling.

Bengtsson (12) designed a microcosm in a column configuration that can be used to study highly volatile compounds or compounds that strongly sorb to rubber or plastic, (Fig. 7.4). A modification of procedures described by Dunlap et al. (13) was used to acquire samples of the deeper subsurface that were not contaminated with surface microbes. A bore hole of the desired depth was drilled with an auger. Then the auger was removed and the sample taken with an autoclaved thin-wall core barrel 9.5 cm I.D. x 45 cm long. The core was extruded 3 to 5 cm, then broken off to produce an aseptic face. The remainder of the core was extended past a device that pared away the outer 1.2 cm of core material. As a result, the core material that was in contact with the core

barrel, and thus might be contaminated with surface microbes, was discarded. The last 4 cm of the core was not extruded to preclude contamination from the extruder. The aseptic sample was extruded directly into a glass column 7.0 cm I.D. x 40 cm long. The column had a sampling port at 20 cm and at the effluent end. A special Teflon well was constructed to provide water from the same lens of sand that was sampled by the core barrel. The water was filter sterilized, then dosed with a suite of organic chemicals and stirred in a sealed container until all compounds were dissolved. The resulting feed solution was stored in flexible Teflon bags supported in a plexiglass box filled with water. The bags had virtually no headspace, which prevented loss of volatile organic compounds from the feed solution. Pressure from a reservoir forced the feed solution onto three replicate columns. Flow through the columns was maintained at 2 cm/day by a peristalic pump downstream of the column effluent port.

Samples for volatile constituents were drawn by connecting a sterile gas-tight syringe to the sample port. Samples for non-volatile constituents were drawn by opening the port and allowing the sample to drip into a vial. All materials touching the feed solution, aquifer matrix material, or samples, were either Teflon or borosilicate glass. All experimental manipulations were performed aseptically. The microcosm was incubated at 17°C, the in situ temperature.

Using this microcosm, Wilson et al. (14) found no evidence of degradation of chloroform, 1,1-dichloroethane, 1,1,1-trichloroethane, trichloroethene, tetrachloroethene, toluene, chlorobenzene or 2-chloroethyl vinyl ether in sandy aquifer material collected just below the water table at a depth of 6.5 m. This is somewhat surprising because chlorobenzene and toluene, at least, are easily degraded in surface soil (15), and the aquifer material contained an

appreciable density of bacteria (10^6/g dry material) as determined by both plate count and direct enumeration using epifluoresence microscopy (16).

This microcosm is very flexible in terms of the nature of the compounds that may be studied. It is also a very faithful simulation of the subsurface environment; it is built with authentic and uncontaminated subsurface material that is exposed to dose water prepared with well water from the same subsurface location, and percolated through the column at hydrologically realistic flow rates. As is the case with most column configurations, the relative breakthrough times of the compounds of interest and some conservative tracer like chloride or tritiated water reveal the extent of sorption of the compound to subsurface solids. After the concentration of compounds in the column efflucent comes to a steady state, a comparison of the concentration of the compounds in the feed solution and column effluent reveals the extent of degradation during passage through the column.

This microcosm is, however, expensive to build and laborious to operate. Most of the labor involves aseptic procedures for preparing feed solutions and acquiring samples. Additionally, it is only suitable for subsurface materials that are relatively permeable, because water must pass through the microcosm. Also the aquifer matrix material must be sufficiently fluid to seal against the walls of the column and prevent channeling or short circuiting of the feed solution. This microcosm would be appropriate for a site study where very trustworthy information is needed about the fate of specific pollutants in a particular subsurface environment. It also is an excellent research tool to evaluate the behavior of simpler microcosms.

Wilson et al. (14) developed a simple microcosm that can also be used to study volatile or reactive compounds (Fig. 7.5). Authentic uncontaminated samples

of the subsurface were obtained as described above (12). Sterile water was mixed into the sample until it became a slurry, then the slurry was transferred to 35-ml screw-cap test tubes. Space was left for approximately 1 ml of dose solution. Sterile dose solution was prepared as described above. The remaining space in the microcosms was filled with dose solution, the tip of the tube was wiped clean with a sterile piece of paper, and the tube sealed with a 25-mm Teflon-lined screw cap. Then the dose solution was dispersed through the slurry with a vortex mixer. A portion of the subsurface material was autoclaved prior to dispensing into the test tubes to provide an abiotic control. The microcosms were sampled by diluting the pore water. The cap was removed and the microcosm was screwed into one end of a large Teflon stopcock with a 10 mm bore. A 35-ml test tube filled with water was screwed into the other end. Then the cock was opened and the contents of the two tubes were mixed together with a vortex mixer. Finally the cock was closed, and the tubes were separated, sealed with Teflon-lined screw caps, and centrifuged gently until the diluted pore water was clear. Microcosms were analyzed after 0, 1, 3, and 9 weeks of incubation. At each time period, at least two microcosms plus an autoclaved microcosm were analyzed.

In samples from immediately above and below the water table from a site at Fort Polk, Louisiana and a site near Pickett, Oklahoma, there was no detectable degradation of chloroform, 1,1-dichloroethane, 1,1,1-trichloroethane, trichloroethene, tetrachloroethene, and chlorobenzene. Styrene and 2-chloroethyl vinyl ether disappeared in all four samples. Toluene disappeared slowly in both samples from Fort Polk, but not in the samples from Pickett. In the autoclaved samples, toluene and styrene did not disappear, suggesting that biodegradation had occurred in the unautoclaved samples. 2-Chloroethyl vinyl ether disappeared in the autoclaved samples, indicating an abiotic process.

This microcosm is well-suited for screening compounds or subsurface materials. It faithfully simulates the subsurface environment with the exception of the flow of water. It can be used with highly volatile or hydrophobic compounds. The microcosms are easy to set up and require no maintenance. They accept materials with a wide range of textures. The experimental manipulations are so simple that the entire process from extruding the core to completion of the microcosm can be carried out in a glove box under a nitrogen atmosphere. As a result, biological processes can be examined in samples from anaerobic subsurface environments that might harbor oxygen-sensitive microbes. The recovery of added compounds from the autoclaved microcosms provides information about aerobic degradation or sorption; although the quality of the data is generally not as good as column-breakthrough data, it is usually adequate for screening purposes.

The microcosm has several limitations. The volume of water available for analysis is small, generally 15 to 30 ml, and the compounds of interest are diluted by a factor of 4 to 8 in the process of sampling. As a result, a sensitive analytical scheme is required to examine the fate of compounds at environmentally relevant concentrations. Additionally, sampling is destructive; thus a fairly large amount of core material is required to set up a properly replicated and adequately controlled assay. Finally, the subsurface material is mixed to a slurry before being loaded into the test-tubes. This destroys much of the spatial heterogeniety present in the sample and may destroy micro-habitats required by certain groups of microbes. Work is underway at the Robert S. Kerr Environmental Research Laboratory comparing the behavior of several organic compounds in both the test-tube microcosm and the glass-column microcosm to determine whether disruption of potential microhabitats is a serious difficulty.

7.5 Microcosms as Physical Models.

Rees and King examined the degradation of phenol in aquifer matrix material under aerobic (17) and anaerobic (18) conditions. Glass columns 4.5 cm I.D. x 60 cm long were packed to a depth of 40 cm with material from the Lower Greensand formation that was collected from a depth of 1.5 m. They were dosed with sterile solutions of phenol. In the case of the aerobic columns, no mineral nutrients were added; in the anaerobic columns, acetate, formate, phosphate, ammonia, and a number of other salts were added to simulate leachate from a domestic-refuse landfill. Apparently aseptic precautions were not taken in collection of the aquifer matrix material or preparation of the columns. Water was collected from positions within the aerobic columns through porous ceramic thimbles. The columns were operated at various flow rates and concentrations of phenol until they came to a steady state. Starting at 50 mg/l., degradation of phenol under aerobic conditions was first order up to approximately 10,000 mg/l. At higher concentrations the phenol was inhibitory. These results are surprising, because Yang and Humphry (19) found that aerobic degradation of phenol by Pseudomonas putida and the yeast Trichosporum cutaneum in culture medium had a K_s in the range of 2 mg/l. Degradation would be essentially zero order at concentrations above 10 mg/l. This is three orders of magnitude lower than the results of Rees and King (17, 18).

Almost all of the phenol-degrading bacteria that could be enumerated by the MPN technique were found in the first 4 cm of the column; the great majority were found in the first 2 cm. However, the first sampling thimble was placed at 6 cm. This situation illustrates one of the primary difficulties with a column configuration. The microcosm is often spatially heterogeneous with respect to degradative activity, although calculation of a rate constant assumes homogeneous conditions over the entire microcosm, or at least over

the interval between sampling ports. As a result, the value of the rate constant determined in a column microcosm can depend on the length of the column.

Degradation of phenol under anaerobic conditions was studied by purging oxygen from the feed solution and from the column headspace with N_2. The columns were inoculated with leachate from a laboratory-scale compost pile of municipal waste. At steady-state, degradation was generally first order at concentrations from 200 to 1,500 mg/l, and zero order above 1,500 mg/l.

Rees and King (17,18) used the Michaelis-Menten equation to extrapolate their laboratory measurements to hypothetical field situations. Such an approach will probably be necessary in order for the science of environmental projection to reach maturity. However, it must be established that the assumptions inherent in the mathematics are met by the subsurface environment being described. For example, the Monod equation assumes that the concentration of organic compound is the limiting factor for its degradation. Frequently, however, degradation in the deeper subsurface will be limited by other requirements for metabolism, particularly oxygen needed for respiration, and phosphorous and nitrogen needed for growth.

7.6 Rational Design of Microcosms.

Microcosm design involves a number of tradeoffs between convenience of operation and/or cost and faithful simulation of the environmental conditions that significantly impact the process of interest. The judgment involved in identifying these important environmental conditions can only come from experience with the larger physical system. Therefore, microcosms should be designed by specialists in the environment being described. If the microcosm is to be used as a physical model, additional constraints are imposed on the experimental design to ensure that data required by the equations are properly collected. The designer must either understand the mathematics or work

closely with someone who does, prior to designing and constructing a microcosm.

Microcosms obey a law of diminishing returns. As microcosms become larger and more complex, the amount of time spent designing, constructing, and maintaining the system seems to increase exponentially. Microcosms should be as simple as possible. In many cases, it is cheaper to build two systems modelling two unrelated phenomena, and optimize design of each system for its particular phenomenon, than to build one common system that does a poor job on both. One useful approach to scaling a microcosm is to first determine a concentration of pollutant to be studied which is environmentally realistic. Then determine the extent of sorption or degradation the microcosm should be able to detect. A change in concentration of at least two orders of magnitude is needed to determine a rate law. Then determine the sensitivity of the analytical instrument. Finally, calculate the quality of sample that must be extracted and concentrated to produce a detectable response on the instrument at the lowest concentration the microcosm is expected to detect. Then scale the microcosm to produce that quanitity of sample.

If at all possible, microcosms should be replicated. Variability in many environmental parameters increases with a decrease in the size of the sample, due in part to stochastic processes. Unless microcosms are replicated, it is impossible to tell if an unexpected result is a statistical aberration, or if it represents the usual behavior of the system.

Additionally, the container and plumbing of the microcosm must not affect the processes under study. The experimenter should demonstrate that such is the case prior to the initiation of a long and complex study. If the system is build of nonreactive materials (e.g., glass, teflon, or stainless steel rather than PVC and Tygon), it is less likely that problems of this nature will

occur. The major cost in most microcosm studies is chemical analysis of the samples, particularly analysis of organic compounds at trace concentrations. It is foolish to try to save a relative small amount of money on the microcosms *per se*, and risk compromising the results of expensive analytical procedures.

7.7 References

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Table 7.1 Variability of conservative ground-water quality parameters in the valley of the Platt River in Nebraska (1).

Depth of well*	Coefficient of Variation: Temperature	Coefficient of Variation: Chloride
	Terrace wells slotted in principal aquifer.	
Shallow	8.3%	26%
	Terrace bottomland well slotted in secondary aquifer.	
Deep	4.6%	26%
	Bottomland wells slotted in principal aquifer and downgradient from irrigated cropland.	
Shallow	28.2%	13%
Deep	6.3%	12%
	Bottomland wells slotted in principal aquifer and influenced by river seepage and near pristine conditions.	
Shallow	43.3%	10%
Deep	5.6%	9.6%
	Platte River.	
No well	72.6%	12.9%

*Shallow and deep refer to wells which were slotted 2 and 18 m or more below the static water level, respectively.

Table 7.2 Variation in solutes in ground water from ingneous rocks throughout the world (2).

Parameter	Range of Means (Data taken from many parts of the world) (pH units and g/m^3)		
pH	5.5	-	7.1
HCO_3^-	2.0	-	59.2
Cl^-	0.6	-	22.0
SO_4^{2-}	0.4	-	10.9
SiO_2	5.9	-	85
Na^+	0.2	-	16.5
K^+	0.6	-	3.4
Ca^{2+}	0.4	-	11.9
Mg^{2+}	0.1	-	4.9

Table 7.3 Survival of bacteria in ground-water microcosms.

Microcosm	Organism	T_{90}* (days)	Reference
Flask	Antibiotic Resistant		4
	E. coli (J 6-2)	2.6	
	(PB 922)	3.0	
	Neotype *E. coli*	3.2	
Dialysis Tubing	Faecal coliforms	6.2	5
Membrane Filter	FROM CULTURE		6
	Coliform bacteria	2.4	
	Enterococci	3.0	
	Streptococcus equinus	1.4	
	S. bovis	0.6	
	Shigella dysenteriae	3.1	
	S. sonnei	3.4	
	S. flexneri	3.7	
	Salmonella enteritidis (ser. paratyphi A)	2.2	
	S. enteritidis (ser. typhimurium)	2.2	
	(ser. paratyphi B)	0.3	
	S. typhi	0.8	
	Vibrio cholerae	1.0	
	FROM RAW SEWAGE		
	Coliform bacteria	2.4	
	Streptococci	2.7	

*Time required for loss of 90% of the original density.

Fig. 7.1 A microcosm to study survival of microorganisms in ground water (6).

Fig. 7.2 A microcosm to study biotransformations of concentrated industrial wastes after deep-well disposal (7).

Fig. 7.3 A microcosm for the study of biotransformations of pollutants which uses internal recirculation of water to allow repeated sampling (11).

Fig. 7.4 A microcosm in column configuration to study biotransformations of pollutants which are highly volatile or sorb strongly to rubber or plastic (12).

Fig. 7.5 A simple microcosm to study biotransformations of pollutants which are highly volatile or sorb strongly to rubber or plastic (14).

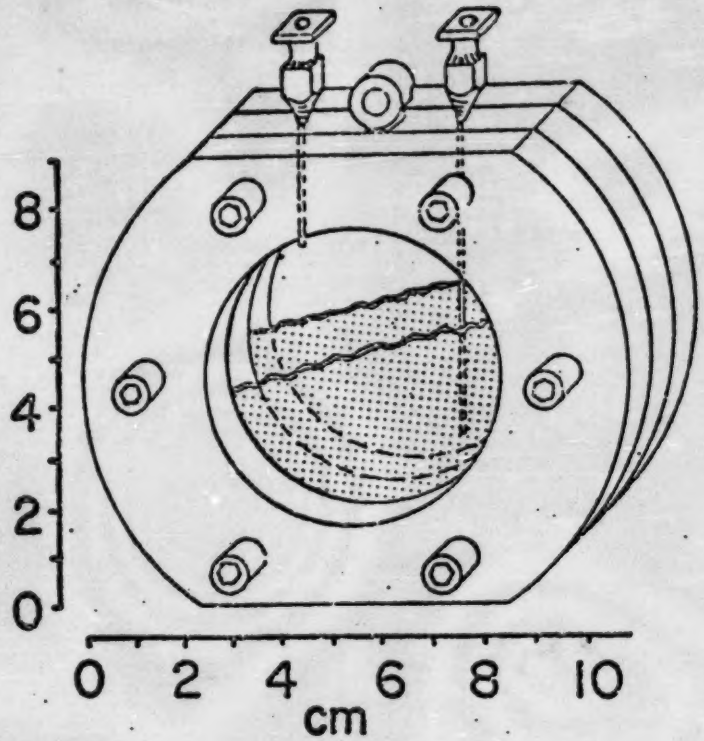

Fig. 7.1 A microcosm to study survival of microorganisms in ground water (6).

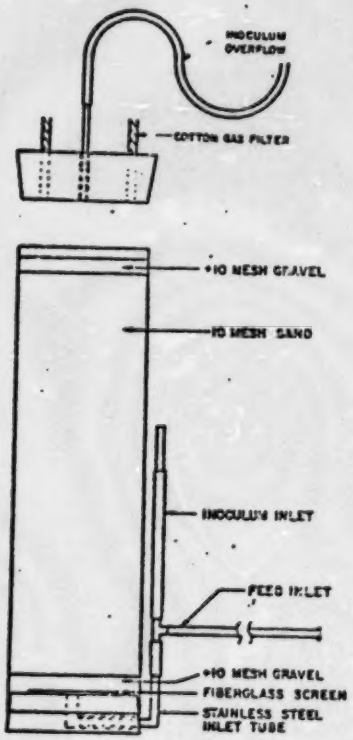

Fig. 7.2 A microcosm to study biotransformations of concentrated industrial wastes after deep-well disposal (7).

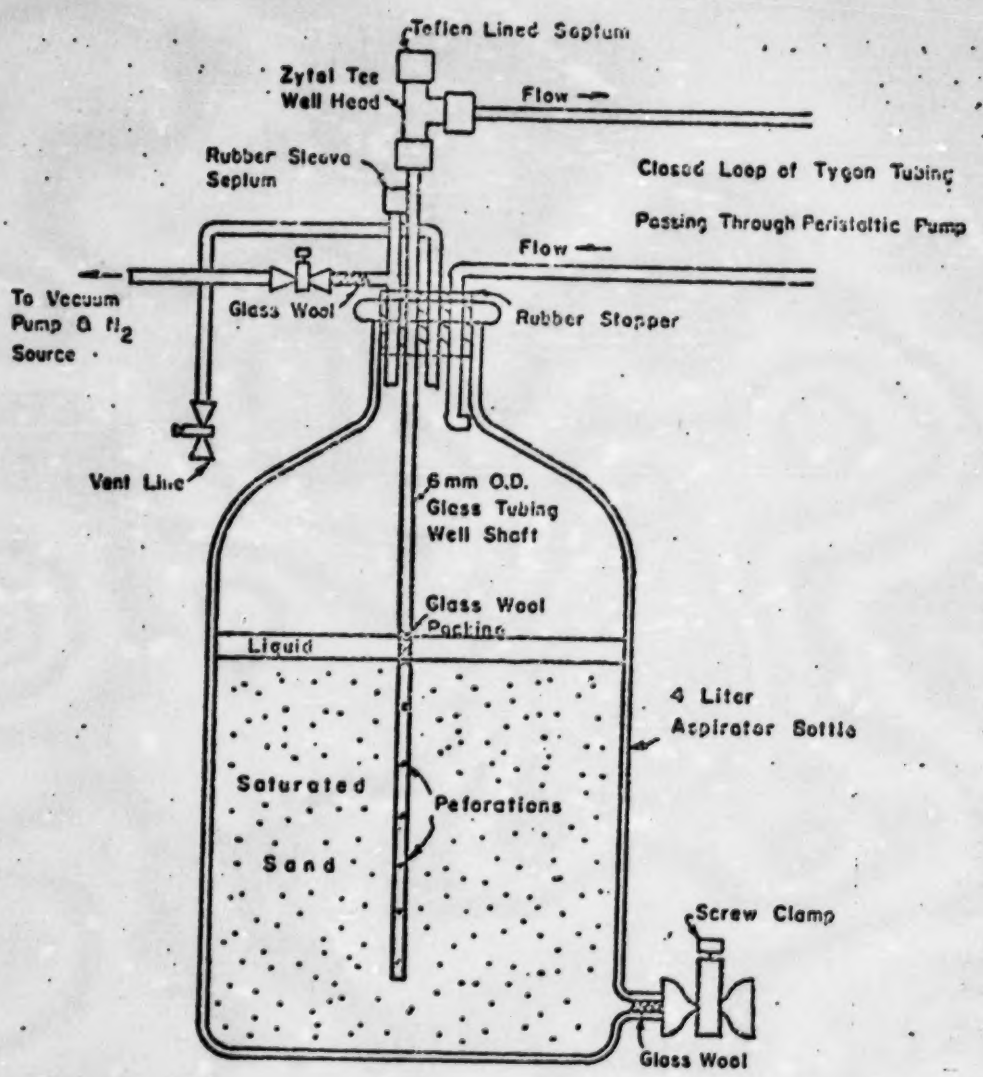

Fig. 7.3 A microcosm for the study of biotransformations of pollutants which uses internal recirculation of water to allow repeated sampling (11).

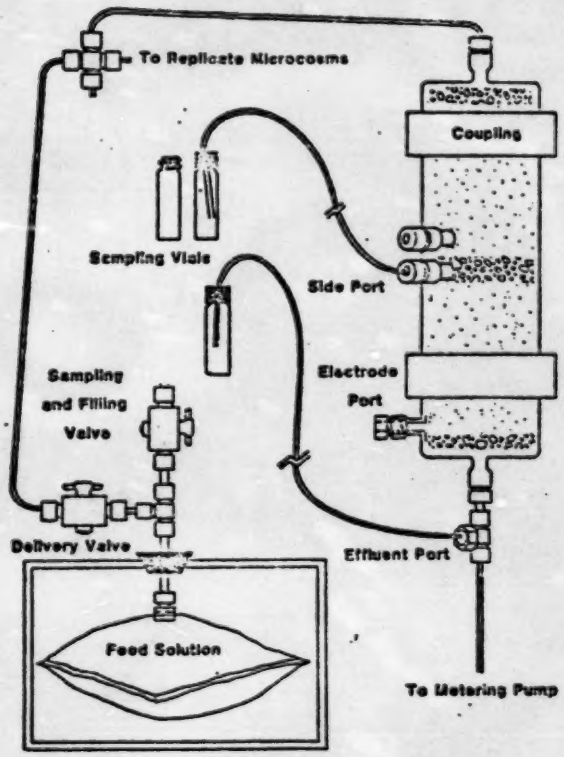

Fig. 7.4 A microcosm in column configuration to study biotransformations of pollutants which are highly volatile or sorb strongly to rubber or plastic (12).

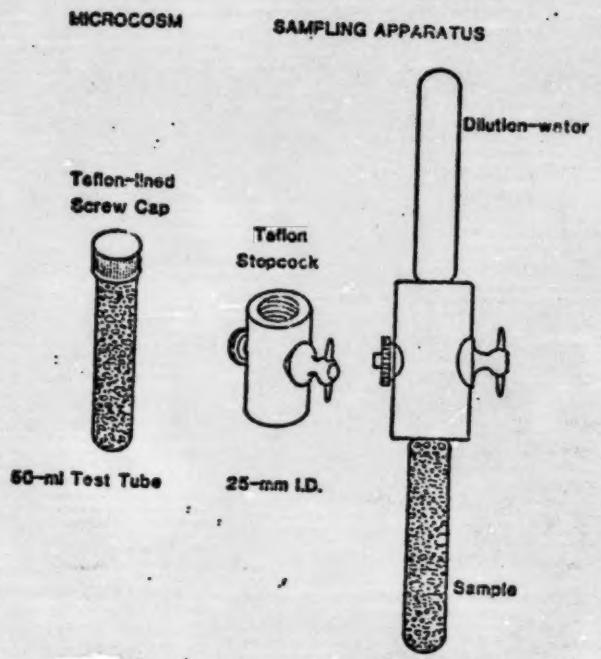

Fig. 7.5 A simple microcosm to study biotransformations of pollutants which are highly volatile or sorb strongly to rubber or plastic (14).